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The role of steroid receptor coactivators in hormone dependent cancers and their potential as therapeutic targets

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Abstract

Steroid receptor coactivator (SRC) family members (SRC-1, SRC-2, SRC-3) interact with nuclear receptors (NRs) and many transcription factors to enhance target gene transcription. Deregulation of SRCs is widely implicated in NR mediated diseases, especially hormone dependent cancers. By integrating steroid hormone signaling and growth factor pathways, SRC proteins exert multiple modes of oncogenic regulation in cancers and represent emerging targets for cancer therapeutics. Recent work has identified SRC-targeting agents that show promise in blocking tumor growth *in vitro* and *in vivo*, and have the potential to function as powerful and broadly encompassing treatments for different cancers.

Introduction

The nuclear receptor (NR) superfamily of transcription factor is composed of ligand-dependent members and orphan receptors whose ligands have not yet been identified or do not have a cognate ligand. The former respond to and transduce hormonal signals into transcriptional programs that drive physiological functions. Decades of research has gained tremendous insight into the actions of NRs on target gene promoters, revealing that they work in close collaboration with coactivators and corepressors to enhance and repress gene transcription, respectively.

The first NR coactivator, steroid receptor coactivator 1 (SRC-1/NCOA1) was identified two decades ago by our laboratory in a yeast two-hybrid screen as a protein that interacts with the ligand binding domain of the progesterone receptor (PR) and it was found to strongly potentiate the transcriptional activities of PR, the estrogen receptor α (ER α) and many other NRs in a ligand-dependent manner (1). SRC-2 (NCOA2/TIF2/GRIP1) and SRC-3 (NCOA3/AIB1/RAC3/ACTR/pCIP) were cloned and characterized shortly afterwards (2-9). These three proteins comprise the most in-depth studied coactivator family. SRC family members share ~60% sequence similarity and contain several conserved structural domains: an N-terminal basic helix-loop-helix-Per/ARNT/Sim (bHLH-PAS) domain, a central NR interaction domain (RID) with three LXXLL motifs, and two activation domains (AD1 and

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AD2) at the C-terminus (10,11). Once recruited to target gene promoters by NRs, SRC proteins can assemble a multi-protein coactivator complex by further recruiting secondary coactivators and histone modifying enzymes such as CBP/p300, coactivator associated arginine methyltransferase 1 (CARM1) and protein arginine methyltransferase 1 (PRMT1) to increase chromatin accessibility for active transcription (6,12-14). From knock-out mouse models, we have learned that although highly homologous to one another, SRC proteins are functionally distinct and that each controls distinct physiological processes, including growth and development, reproduction and energy homeostasis (10,15). As our knowledge of the biology of SRC proteins improves, it is clear now that these proteins undergo heavy post-translational modifications which dictate their preference for transcription factors and alter their protein stability and transcriptional activity (16,17). This has revealed multiple layers of complexity to the transcriptional programs controlled by SRC proteins leading to the realization that SRCs are key integrators of extracellular environmental cues and upstream signaling pathways with cellular transcriptional programs.

Due to their strong association with NRs, SRC proteins have been found to be intricately involved in the physiological and pathological processes in which these receptors are implicated. For instance, SRC proteins are fundamental in the proper functions of steroid hormones; abnormal changes that lead to altered SRC expression or activity can be a driving force in the initiation and progression of metabolic diseases, reproductive diseases and especially cancers in hormone-sensitive tissues. This review will focus on the roles of SRC proteins in hormone dependent cancers and their therapeutic potentials.

Breast cancer

Ever since its initial identification, SRC-3 has been recognized as a very prominent player in breast cancer. The gene encoding SRC-3 is amplified at a frequency of approximately 5%-10% in breast cancers (5,18), while SRC-3 mRNA or protein has been found to be overexpressed in up to 60% of cases in different breast cancer cohorts (5,19,20). Clinically, SRC-3 overexpression in breast cancer correlates with larger tumor size (18), higher tumor grade (21) and poor survival rates (22). Both *in vitro* and *in vivo* breast cancer models have provided valuable information to help us understand SRC-3's role in breast cancer. SRC-3 has been shown to mediate estrogen-dependent breast cancer cell proliferation and survival (23,24). *In vivo*, loss of SRC-3 significantly reduces breast tumor incidence, and delays tumor growth and development in breast cancer mouse models driven by oncogenes (i.e. *MMTV-v-ras*, *MMTV-ErbB2*) (25,26) or induced by chemical carcinogens (27). In these models, insulin-like growth factor 1 (IGF1) and ERBB2 signaling pathways are impaired in the absence of SRC-3. Direct evidence supporting the oncogenic role of SRC-3 comes from the *MMTV-SRC-3* transgenic mouse model, which shows that overexpressing SRC-3 is sufficient to cause spontaneous development of malignant mammary tumors as well as tumors in other organs including the pituitary and uterus (28). Furthermore, genetic ablation of SRC-3 in the *MMTV-PyMT* mice dramatically reduces breast cancer metastasis to the lung (29). Molecular analysis reveals that SRC-3 coactivates the Ets transcription factor polyoma enhancer activator 3 (PEA3) to promote the expression of matrix metalloproteinase 2 (MMP2) and MMP9. Interestingly, the expression of SRC-3 in human breast tumors positively correlates with that of PEA3, MMP2 and MMP9. A similar report from a study

using MDA-MB-231 breast cancer cells shows that SRC-3 regulates activating protein -1 (AP-1)-driven MMP7 and MMP10 expression (30). These findings demonstrate that SRC-3 is a critical pro-metastatic factor in breast cancer that also upregulates MMP expression to promote cancer cell invasion. Clinical data from breast cancer patients with or without tamoxifen treatment indicate that high SRC-3 expression is associated with worse disease free survival, implicating SRC-3 in therapy resistance (31).

Intriguingly, a splice variant of SRC-3 (SRC-3^{Δ4}) was recently demonstrated as a bridging adaptor between the epidermal growth factor receptor (EGFR) and focal adhesion kinase (FAK) that potentiates cancer cell migration and invasion in a non-genomic manner (32). Lacking the bHLH-PAS domain which contains the nuclear localization sequence, SRC-3^{Δ4} is mainly localized in the cytosol. Upon cancer cell exposure to EGF, SRC-3^{Δ4} is phosphorylated by p21-activated kinase 1 (PAK1), which triggers its translocation to the plasma membrane where it binds to both EGFR and FAK, allowing signal transduction and full activation of this signaling cascade essential for cancer cell invasion and metastasis.

Expression of SRC-1 also is significantly increased in about 20% of breast cancers, and is positively correlated with ERBB2 expression, disease recurrence and poor survival (33-35). SRC-1 is overexpressed in aromatase inhibitor-resistant breast tumors (36). Although SRC-1 promotes estrogen-dependent breast cancer cell growth and proliferation *in vitro* (37,38), in an *MMTV-PyMT* breast cancer mouse model, SRC-1 deficiency does not affect tumor initiation and growth, but drastically inhibits lung metastasis (39). Mechanistic studies have shown that SRC-1 promotes metastasis by regulating the expression of colony stimulating factor 1 (CSF1) which recruits tumor associated macrophages (39), and by coactivating PEA3-mediated expression of TWIST1, a master regulator of epithelial-mesenchymal-transition (EMT) (40).

Despite being reported to mediate estrogen-induced breast cancer cell proliferation and target gene expression *in vitro* (41), compared with SRC-3 and SRC-1, the role of SRC-2 in breast cancer has been less well characterized.

Prostate cancer

A comprehensive sequencing analysis of human prostate tumors, prostate cancer cell lines and xenografts has shown that 8% of primary and 37% of metastatic tumors have gain-of-expression (overexpression and amplification) of the SRC-2 gene (42). Primary tumors with SRC-2 amplification display increased androgen receptor (AR) signaling based on their AR target gene signature, consistent with the known role of SRC-2 as an AR coactivator. Moreover, castrate patients with primary tumors harboring SRC-2 mutations, overexpression or amplification have higher rates of recurrence (42). These findings are in agreement with previous reports about the positive correlation between SRC-2 expression and high tumor grade and poor survival (43-45). Hence, SRC-2 has been proposed as a driver oncogene in primary prostate tumors. Since SRC-2 has long been recognized as a master regulator of energy homeostasis (46-49), a recent study from our laboratory probed the functional significance of SRC-2 upregulation in prostate cancer metabolic programming and found that SRC-2 can drive glutamine-dependent and sterol regulatory element binding protein 1

(SREBP1)-mediated *de novo* lipogenesis, supporting prostate cancer cell survival and metastasis (50).

SRC-3 has been reported to be overexpressed in prostate cancers and its expression level is positively correlated with tumor grade and disease recurrence (51). SRC-3 not only is required for prostate cancer cell proliferation and survival (52,53), but also promotes invasion and metastasis by activating FAK and focal adhesion turnover as well as by increasing the expression of MMP2 and MMP13 (54). A high level of SRC-1 also is found in recurrent prostate cancer (45), and it has been shown in cell culture to promote AR function (55).

Endometrial cancer and ovarian cancer

As coactivators of ER α and PR, roles for SRC family proteins have been investigated in malignancies in the endometrium and ovary. It has been reported that the mRNA levels of all three SRCs are significantly increased in endometrial carcinoma (56). In particular, SRC-3 expression is correlated with clinical stage, depth of myometrial invasion and differentiation, as well as poor prognosis (57,58).

Even at the time of its discovery, the SRC-3 gene was found to be amplified in ovarian cancer (5,18). Additionally, upregulation of SRC-3 has been observed in 64% of high-grade ovarian cancers, and its level is positively associated with disease severity (59). Interestingly, independent of ER α status, SRC-3 is required for the correct cellular localization of FAK in ovarian cancer cells, and experimental disruption of SRC-3 expression blocks ovarian cancer cell motility and migration (59).

SRC family proteins as therapeutic targets

As coactivators of steroid NRs, aberrant overactivation or overexpression of SRC family proteins greatly enhances steroid hormone signaling, giving a competitive edge for cancer to develop in hormone sensitive organs. Moreover, the ability of SRC proteins to coactivate many other transcription factors such as NF- κ B (60), SREBP1 (50), PEA3 (29,40) and AP-1 (30,54) extends their impact to more pathways pertinent to cancer cells. Importantly, SRC proteins can be phosphorylated and activated by various kinases, so that they can receive extracellular signals and convert them into distinct and enhanced transcriptional outputs crucial for cancer cell proliferation, survival and metastasis (Fig. 1). For instance, seven Ser/Thr (Thr24, Ser505, Ser543, Ser601, Ser857, Ser860 and Ser867) and one Tyr (Tyr1357) phosphorylation sites on SRC-3 have been identified, which are targeted by different kinases such as MAPK, IKK, GSK3, PKA, casein kinase 1 isoform δ (CK1 δ) and ABL, under the stimulation of steroid hormones or growth factors, resulting in increased SRC-3 coactivator function and gene transcription (61-63). Similarly, phosphorylation of Thr1179 and Ser1185 on SRC-1 by IL-6 signaling or MAPK augments its affinity for NRs and leads to stronger NR-dependent transcription (64,65). SRC-2 also has been reported to be activated by phosphorylations on Ser469, Ser487, Ser493, Ser499, Ser699 or Ser736 mediated by casein kinase (CK), cyclin-dependent kinase 9 (CDK9), MAPK or mTOR (50,66-69). Therefore, SRC proteins are central integrators and promoters of multiple signaling pathways, which

makes them key targets for future cancer drug development. Because inhibiting SRCs can simultaneously interfere with many pathways, the chance of cancer cells to develop resistance and evade therapy to these agents is expected to be significantly reduced.

Lacking a structurally defined enzymatic activation domain or a high affinity ligand binding domain, SRC proteins have not been the focus of current drug development which has largely been restricted to a small number of proteins. However, given the key roles of SRCs in tumorigenesis, the difficulty in their targeting is outweighed by the potential therapeutic benefits. Efforts have been invested to identify compounds which can disrupt the binding of SRC family members to NRs such as ER α , ER β and PPAR γ (70-72). In a proof-of-principle study, our laboratory initially identified gossypol as a small molecule inhibitor (SMI) of SRC-1 and SRC-3 which can target these coactivators for degradation and cause cell death in various cancer cell lines, for the first time establishing that SRC proteins can be targeted by small molecule compounds (73).

A subsequent high-throughput screening of a chemical library containing compounds from the large NIH-Molecular Libraries Probe Production Centers Network (MLPCN) was carried out by our laboratory to identify inhibitors of SRCs' transcriptional activity. This study led to the identification of improved SRC SMIs, including bufalin and verrucarin A (74,75). Bufalin promotes the degradation of SRC-3 and SRC-1 in a proteasome-dependent manner and efficiently blocks cancer cell growth *in vitro* and *in vivo*. A water soluble analog, 3-phospho bufalin, was developed to overcome the solubility problem of bufalin, and it was proved to be equally efficient in inhibiting tumor growth in an orthotopic breast cancer model (76). Verrucarin A also selectively degrades SRC-3, while affecting SRC-1/-2 to a lesser extent, and blocks cancer cell proliferation and migration. Compared with gossypol, bufalin and verrucarin A are more potent SRC SMIs, exerting strong effects at low nanomolar concentrations. Unlike gossypol and bufalin, which directly bind to the RID of SRC-3, verrucarin A does not physically interact with SRCs. Gossypol, bufalin and verrucarin A are structurally unrelated compounds originally identified for different purposes: gossypol is a natural polyphenol found in cotton seeds which once was pursued as a male contraceptive agent; bufalin is a cardiac glycoside; verrucarin A belongs to a group of sesquiterpene found in toxins of pathogenic fungus, and yet they all can unexpectedly induce SRC protein degradation, implying that ample sources and opportunities exist to identify additional SRC SMIs.

Interestingly, a small molecule stimulator (SMS) of SRCs named MCB-613 also was indentified in the MLPCN high-throughput screen (77). MCB-613 hyper-stimulates SRCs' transcriptional activity, efficiently kills cancer cells and inhibits tumor growth in a breast cancer xenograft mouse model. Molecular analysis revealed that MCB-613 increases SRCs' interaction with other coactivators such as CBP and CARM1, and markedly elevates the intracellular levels of reactive oxygen species (ROS) which is coupled to the strong induction of endoplasmic reticulum stress. Multiple lines of evidence indicate that phosphorylation plays an important role in the activation of SRC-3 by MCB-613: 1) MCB-613 treatment induces phosphorylation on SRC-3; 2) MCB-613 promotes the interaction of SRC-3 with ABL kinase; 3) ABL kinase inhibitors significantly impair the activation of SRC-3 by the compound (77). However, whether ABL is the only kinase

involved and which sites on SRCs are phosphorylated still remains an open question. The identification of MCB-613 has opened up a new avenue to target SRCs in cancer treatment. Considering the oncogenic roles of SRCs, it appears to be counterintuitive to employ SRC SMS in treating cancer. However, in comparison with normal cells, cancer cells are under high levels of stress from increased protein synthesis/folding and metabolism due to their highly proliferative nature. It is of utmost importance for them to fully engage their stress response pathways in order to maintain homeostasis, making them more vulnerable than normal cells to stressors such as MCB-613. By acutely over-stimulating SRC family proteins, MCB-613 overloads the stress response system of cancer cells and selectively kills them. Thus, targeting the SRC coactivators, either by inhibition or stimulation, represents a novel and promising approach in cancer therapeutic development (Fig. 2).

Conclusion

Transcriptional coactivators are indispensable for NRs and transcription factors to regulate target gene expression and carry out important physiological functions. Aberrant activity or expression of SRC proteins are found in pathological conditions, and most prominently, in cancers. By integrating and promoting growth signaling pathways upon which cancer cells rely, SRC proteins represent emerging targets for cancer therapeutics and several SRC SMIs or SMS have been identified that show considerable promise in breast cancer mouse models. Although this review focuses on hormone dependent cancers, SRC proteins have been found to be overexpressed in many other cancer types (10). Therefore, one can foresee that these SRC-targeting drugs could be proved to be widely useful for cancer treatment. Future efforts should be directed not only to broadening our understanding of this group of proteins but also to delivering effective and safe coactivator-targeting agents for clinical use.

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Fig. 1.

SRC family proteins are integrators of multiple signaling pathways crucial for tumor progression. SRC proteins partner with different NRs and transcription factors in the nucleus to activate target gene transcription important for cancer cell proliferation, growth, survival and metastasis. SRCs also promote the oncogenic ERBB2 and IGF1 pathways. SRCs can be regulated by extracellular signals through post-translational modification (PTM), so that they can generate transcriptional outputs in a signal-dependent manner. SRC-3 4, an SRC-3 splice variant, can mediate signal transduction from EGFR to FAK in the cytosol and enhance the migratory potential of cancer cells

Fig. 2.

SRC proteins can be targeted by small molecule compounds. The identified SRC SMIs (gossypol, bufalin and verrucarin A) can degrade SRC proteins, leading to decreased proliferation and increased apoptosis. MCB-613, an SRC SMS, can hyper-activate the transcriptional activities of SRC proteins and cause cancer cell-specific cytotoxic stress. Although mechanistically different, both of these two classes of chemicals can efficiently kill cancer cells and block tumor growth